Golgi Complex:- 1. Meaning of Golgi Apparatus 2. Occurrence of Golgi Apparatus 3. Location 4. Structure 5. Functions.

Meaning of Golgi Apparatus:

Golgi complex (Golgi Apparatus, Dalton Complex, Apparato Reticulare) is a complex cytoplasmic structure made up of smooth membrane saccules or cistemae, a network of tubules with vesicles and vacuoles, which takes part in membrane transformation, secretion and production of complex biochemicals.

It is surrounded by an organelle free cytoplasm called zone of exclusion or Golgi ground substance. It was first seen by George (1867) but is named after Italian scientist Camillo Golgi, who in 1898 recognised the apparatus as reticular structure (apparato reticulare) near the nucleus.

In the nerve cells of barn owl and cat by means of metallic impregnation method. Its structure was studied under electron microscope by Dalton and Felix (1954).

Occurrence of Golgi Apparatus:

Golgi apparatus or complex is absent in prokaryotic cells (PPLO, bacteria and blue-green algae). It is present in all eukaryotic cells except sieve tubes of plants, sperms of bryophytes and pteridophytes and red blood corpuscles of mammals.

Location of Golgi Apparatus:

In animal cells Golgi complex or apparatus is either single or consists of a single connected complex. The two conditions are respectively called localised (most vertebrate cells) and diffused (most invertebrate cells, liver and nerve cells of vertebrates).

The localised organelle is compact. It generally occurs at one end between the nucleus and the periphery. The diffused organelle is found to form a network, e.g., around the nucleus in nerve cells.

In plant cells, Golgi apparatus is formed of a number of unconnected units called dictyosomes. Their number is highly variable— from one in certain simple algae to 25000 in rhizoidal

cell of Chara. Commonly there are 10-20 dictyosomes per plant cell. A liver cell may possess up to 50 units of Golgi apparatus called Golgisomes.

Structure of Golgi Apparatus:

The shape and size of Golgi complex are not fixed. They depend upon the physiological state of the cells. A typical plant dictyosome is 0.5-1.0 pm in diameter. Usually Golgi complex is made up of four parts— cisternae, tubules, vesicles and vacuoles (Fig. 8.32).

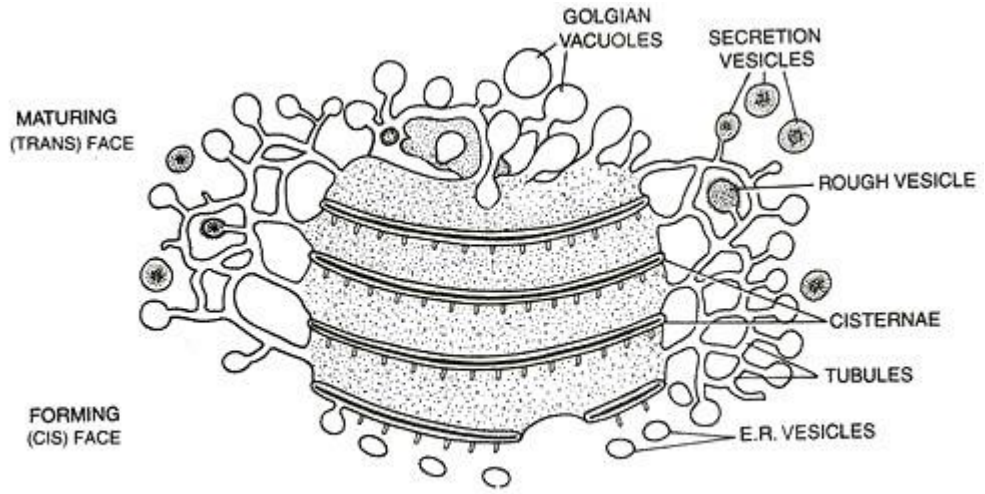

Fig. 8.32. Structure of Golgi apparatus (dictyosome)

Cisternae:

Golgi complex consists of a stack of generally 4-8 (range 3-20) membrane bound saccules or cisternae. Unicistemal dictyosomes are found in fungi.

The membranes of the saccules or cisternae are smooth but of variable thickness they enclose a lumen of 60-90 A. Lumen contains a fluid substance or matrix. In a stack, the adjacent cisternae are separated by a distance of 100-300 A. The intercisternal space contains thin layer of cytoplasm having parallel fibrils.

The saccules are frequently curved to give a definite polarity to the Golgi apparatus. One face of the apparatus is convex while the other is concave. The convex side is called forming (=formative, cis-face) face while the concave side of the apparatus is known as maturing face (trans-face). The membranes of the maturing face are 7-8 nm in thickness while those of the forming face are about 4 nm in thickness.

The forming face receives (transitional) vesicles from endoplasmic reticulum. Their contents pass through various cisternae with the help of coated vesicles and intercisternal connectives. They ultimately reach the maturing face where they are budded off as secretion, coated or Golgian vesicles or vacuoles. While passing through the apparatus, bio-chemicals are variously transformed.

TUBULES

They form a complicated network towards the periphery and maturing face of the apparatus. Actually tubules arise due to fenestrations of the cisternae. They have a diameter of 30-50 nm. The tubules interconnect the different cisternae.

Vesicles:
They are small sacs of 20-80 nm diameters. The vesicles are found attached to the tips of tubules at various levels in the network. They are of two types, smooth and coated. The coated vesicles have a rough surface. They elaborate membrane proteins. The smooth vesicles have a smooth surface. They contain secretory substances and are hence known as secretion vesicles.

Golgian Vacuoles:
They are expanded parts of the cisternae which have become modified to form vacuoles. The vacuoles develop from the concave or maturing face. Golgian vacuoles contain amorphous or granular substance. Some of the golgian vacuoles function as lysosomes.

Functions of Golgi Apparatus:

1. Secretion:
All glandular cells depend upon Golgi complex for concentrating and packaging their products inside a soluble protein coat visible as dark staining under electron microscope. They are sent out of the cells through exocytosis or reverse pinocytosis.

2. Transformation of Membranes:
Golgi complex brings about membrane transformation, that is, converting one type of membrane (e.g., that of ER) into other types (e.g., selectively permeable plasma membrane, differentiated

membrane of lysosome). The complex also takes part in the recycling of plasma membrane.

3. Glycoproteins and Glycolipids:
Proteins synthesised by the rough endoplasmic reticulum and lipids synthesized by smooth endoplasmic reticulum reach the cisternae of the Golgi apparatus. Here, they combine with carbohydrates to form glycoproteins and glycolipids.

4. Special Simple Carbohydrates:
Sialic acid and galactose are made inside Golgi complex.

5. Complex Carbohydrates:
Most of the complex carbohydrates, other than glycogen and starch, are synthesized inside the Golgi complex, e.g., pectic compounds, mucopolysaccharides, hyaluronic acid, chondroitin sulphate, hemicelluloses, etc.

6. Hormones:
Production of hormones by endocrine glands is mediated through it.

7. Matrix:
Matrix of connective tissue is formed by Golgi complex of its cells.

8. Fat Transport:
Fatty acids and glycerol absorbed by intestinal epithelium are transferred as fat to lacteal through Golgi complex.

9. Synthesis of Pigments:
In Chick embryo the retinal pigment has been observed to be synthesized by Golgi complex.

10. Formation of Acrosome:
Acrosome is an important constituent of the tip of animal sperms which helps in digesting away the covering sheath of the egg or ovum during fertilization. It is synthesized by Golgi complex with the help of its vesicles.

11. Vitellogenesis:

In oocytes of animals, Golgi apparatus functions as the centre around which yolk is deposited. The process is called vitellogenesis.

12. Root Hair:
The formation of root hair from their mother cells is believed to take place through the agency of Golgi apparatus.

13. Formation of Lysosomes:
Some of the vesicles or vacuoles of the Golgi apparatus store digestive enzymes obtained through ER in the inactive state. They act as primary lysosomes.

14. Hypnotoxin:
Hypnotoxin of nematoblasts is formed by Golgi apparatus.

15. Formation of Plasma-lemma:
Membranes of the vesicles produced by Golgi apparatus join in the region of cytokinesis to produce new plasma-lemma.

16. Formation of New Cell Wall:
Pectic compounds of middle lamella and various polysaccharides of the cell wall are secreted by Golgi complex. They are brought to the area of new wall synthesis by secretion vesicles.

Endoplasmic Reticulum: Structure, Types and Function of Endoplasmic Reticulum

The electron microscope reveals an extensive membrane system in the cytoplasm called Endoplasmic reticulum (ER).

It was first reported by Porter in 1945. This continuous membrane system connects the nuclear membrane on one end and the cell membrane on the other.

All cells do not have Endoplasmic reticulum. It is generally absent in egg and embryonic cells. Generally, ELECTRONS is fully developed in cells actively engaged in protein and hormone

synthesis. Endoplasmic Reticulum contains three different types of structure.

These are cisternae, vesicles and tubeless:
(a) Cisternae:
These are long, flat and un-branched plates or lamellae arranged in parallel rows.

(b) Vesicles:
They are usually round or ovoid sacs. They often occur isolated in the cytoplasm.

(c) Tubules:
They are irregularly branched tube-like structures having a diameter of 50-100n. These are surrounded by this unit membrane of 50-60 thickness and their lumen is filled with the secretary products of the cell.

There are two types of ER, such as smooth walled and rough walled.

They may be present in the same or different types of cells namely:
(i) Smooth Endoplasmic Reticulum (SEE):
The surface of this type of reticulum is smooth as ribosome's not attached to it. Smooth ER is actively engaged in steroid synthesis, carbohydrate metabolism, pigment production etc. in cells, (u) Rough Endoplasmic Reticulum: The rough ER have ribosome attached throughout the surface. These are present in cells, which are active in protein synthesis.

The major functions these are the following:
(a)Common to both Endoplasmic Reticulum:
(i) Forms the skeletal framework.

(ii) Active transport of cellular materials.

(iii) Metabolic activities due to presence of different enzymes.

iv) Provides increased surface area for cellular reactions.

(v) Formation of nuclear membrane during cell division.

(b) Function of Smooth Endoplasmic Reticulum:
(i) Lipid synthesis.

(ii) Glycogen synthesis.

(iii) Steroid synthesis like cholesterol, progesterone, testosterone, etc.

(iv) Metabolism of carbohydrates

(v) Detoxification function.

(vi) Major storage and released site of inter cellular calcium ions.

(c) Function of Rough Endoplasmic Reticulum:
(i) It provides site for protein synthesis.

(ii) Protein translocation, folding and transport of protein.

(iii) Glycosylation (this is the relation of a saccharides group with a hydroxyl or amino functional group to form a glucoside).

iv) Disulfide bond formation (disulfide bonds stabilize the tertiary and quaternary structures of many proteins).

(v) Membrane synthesis.

LYSOSOMES

Meaning of Lysosomes in Cell:

Lysosomes are ultra structural particles of the cell containing hydrolytic enzymes responsible for digestion. Though these are common in animal cell, but in plants these are found in the lower groups, such as euglenoids, slime moulds and some saprophytic fungi.

Particles isolated from tobacco and maize seedlings contain several of the hydrolases found in animal lysosomes. They are so called because they contain lytic or destructive enzymes. The enzymes, if released, can digest the cell and hence lysosomes are sometimes called 'suicide bags'.

Structure of Lysosomes in Cell:

Lysosomes are globular or granular in appearance of 0.2-0.5μm size without any characteristic shape or structure. These are bounded by a single lipoprotein membrane containing enzymes in crystalline form. The enzymes present are phosphatase, nuclease, lipase, protease, glycosidase, sulfates, amylase.

The membrane is impermeable to substrates of the enzymes contained in the lysosome. Certain substances, called labializes, cause instability of the lysosomal membrane, leading to release of enzymes from the lysosome. Other substances, called stabilizers, have a stabilizing action on the membrane. This prevents uncontrolled digestion of the cell contents and thus protects the cell from autolysis.

The lysosomes show polymorphism in different cell types. There are two basic types of lysosomes. Golgi complex buds off primary lysosomes containing hydrolytic enzymes. The vacuole or phagosome arising by endocytosis associates and fuses with primary lysosome to form secondary lysosome (Fig. 2.61).

Incomplete digestion results in residual bodies. Lysosomes sometimes include intracellular part like mitochondria or endoplasmic reticulum for digestion are called auto-phagosome.

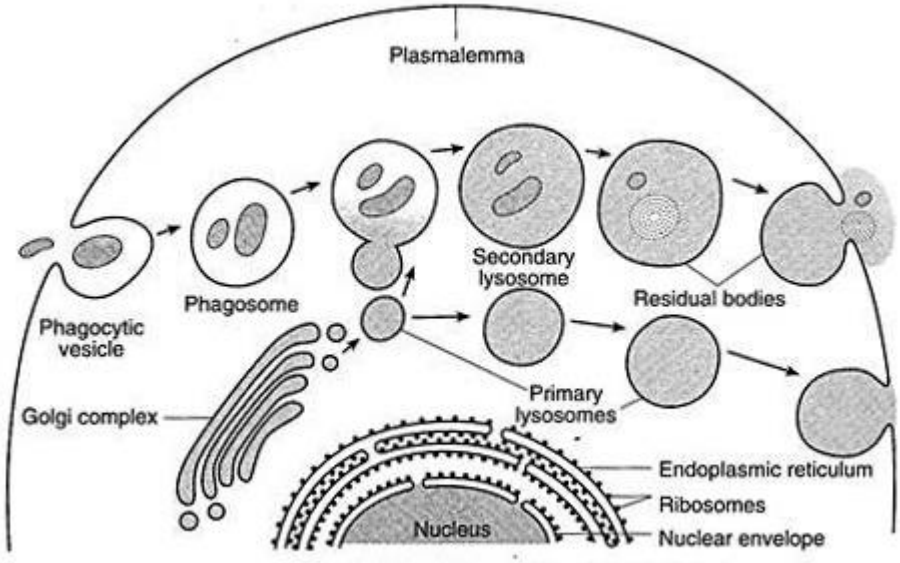

Fig. 2.61: Diagram showing the origin and different phases of lysosomes

Function of Lysosomes in Cell:

Lysosomes are bags containing digestive enzymes involved in digestion of intracellular and extracellular particles. The primary lysosomes secrete hydrolases outside by exocytosis, resulting in degradation of the extracellular material (extracellular digestion). Saprophytic fungi utilize extracellular digestion for nutrition.

Digestion of substances within the cell is called intracellular digestion. Intracellular digestion may involve heterophagy or autophagy. Heterophagy is the intake of exogenous material into the cell by endocytosis and the subsequent breakdown of this material by enzymes in secondary lysosomes. Autophagy brings about digestion of cell's own material on the death of a cell, or with cellular injury.

PEROXISOMES:

They are micro bodies which contain enzymes for peroxide biosynthesis. Peroxisomes were discovered by De Duve (1965) with the help of fractionation technique. The term was coined by De Duve in 1969. Peroxisomes are found in both plant and animal cells, generally in close association with endoplasmic reticulum, mitochondria and chloroplasts.

Despite absence of DNA, peroxisomes are believed to be able to replicate like mitochondria and plastids.

They are believed to vestige of an ancient organelle present in protoeucaryotes which performed all oxidation reactions prior to evolution of mitochondria. They contain special docking proteins called peroxins for obtaining materials from cytosol and endoplasmic reticulum. Peroxisomes occur in all eukaryotic cells.

They are quite abundant in liver and kidney cells. A photosynthetic cell may have 70- 100 peroxisomes. Peroxisomes are believed to develop from endoplasmic reticulum.

Their size and shape are variable. Commonly the peroxisomes have a diameter of 0.5-1.0 μm. They are covered over by a single membrane. The interior contains a matrix which may be granular or have fibrils arranged variously. In some cases the matrix has a central dense, crystalline or fibrous core which is called nucleoid.

The peroxisomes contain oxidative enzymes like urate oxidase, D-amino acid oxidase, a- hydroxy acid oxidase and (3-hydroxy acid oxidase. Molecular oxygen is required. The reactions produce hydrogen peroxide which is immediately metabolized by another enzyme called catalase.

(a) In animal cells, peroxisomes metabolize in number of toxic substances like nitrite, phenols, formaldehyde, formic acid, methanol, ethanol etc. 25% of alcohol consumed by a person is detoxified inside liver cells.

(b) Unusual substances or xenobiotic (e.g., D-amino acids, alkanes) which cannot be metabolized by normal enzymes are broken down inside peroxisomes.

(c) Urate produced during catabolism of nucleic acids and some proteins is changed into allontoin inside peroxisomes.

(d) Long chain (e.g., prostaglandins) and branched chain fatty acids are initially broken down by peroxisomes.

(e) In root nodules, they convert fixed nitrogen in ureids for transport.

(f) Plant peroxisomes found in photosynthetic cells, perform photorespiration.

For this, they are associated with chloroplasts and mitochondria. Peroxisomes pick up glycolate from chloroplasts. The same is oxidized with the help of oxygen to produce glyoxylate. Hydrogen peroxide is formed as by-product. Glyoxylate is changed to amino acid glycine. The glycine condenses to produce amino acid serine and carbon dioxide

Like other micro bodies, glyoxysomes have a single covering membrane and an enzyme rich matrix with a crystalloid core, p-oxidation of fatty acids produces acetyl CoA. The latter is metabolized in glyoxylate cycle to produce carbohydrates.

After completion of their function, glyoxysomes are believed to be changed into peroxisomes. They reappear in senescent plant tissues for degradation of lipids and mobilisation of degradation products.

GLYOXYSOMES:

Glyoxysomes are micro bodies which contain enzymes for (5-oxidation of fatty acids and glyoxylate pathway. They are considered to be special peroxisomes. The micro bodies appear transiently in germinating oil seeds and the cells of some fungi till the stored fat is consumed.

Like other micro bodies, glyoxysomes have a single covering membrane and an enzyme rich matrix with a crystalloid core, p-oxidation of fatty acids produces acetyl CoA. The latter is metabolized in glyoxylate cycle to produce carbohydrates.

After completion of their function, glyoxysomes are believed to be changed into peroxisomes. They reappear in senescent plant tissues for degradation of lipids and mobilisation of degradation products.

Ribosomes of prokaryotic Cell:

Ribosome is a key component in the process of translation therefore studied extensively. One bacterial cell contains ~10,000 such structures and a eukaryotic cell contains many times more.

A bacterial ribosome is about 250 nm in diameter and consists of two subunits, one large and one small. Both subunits consist of one or more molecules of rRNA and an array of ribosomal proteins.

Association of two subunits is called mono-some. The structure of prokaryotic ribosome is given in the figure 8.2 B. The subunit and rRNA components are most easily isolated and characterize on the basis of their sedimentation behavior in sucrose gradient (there rate of migration is called Svedberg's coefficient 'S'.

It is a unit of sedimentation velocity; sedimentation in ultracentrifuge depends on both the mass and shape of molecule, and is not a simply a measure of molecular mass) (Fig. 8.2A). The mono-some of prokaryotes is a 70S particle and in eukaryotes it is 80S. The prokaryotic 70S mono-some consists of a 50S and 30S subunit and the eukaryotic 80S mono-some consists of a 60S and 40S subunit. The sum value of 'S' is not a simple arithmetic addition.

The large subunit in prokaryotes consists of a 23S RNA molecule, a 5S rRNA molecule and 31 ribosomal proteins. In the eukaryotic equivalent, a 28S rRNA molecule is accompanied by a 5.8S and 5S rRNA molecule and ~50 proteins. The smaller prokaryotic subunits consist of a 16S rRNA component and 21 proteins. Similarly, in eukaryotes smaller subunits consist of a 18S rRNA component and ~33 proteins. These proteins are involved in binding of various molecules involved in translation and precise control of process.

In eukaryotes, many more copies of a sequence encoding the 28S and 18S components are present. The exact functions of various components are still not very much clear. In bacteria, the mono-some has a combined molecular weight of 2.5 million Dalton.

Structure of tRNA is described in Chapter 5. Transfer RNA has an anticodon loop and a amino acid binding site besides other loops and stems as described earlier. Before translation can proceed, the tRNA molecule must be chemically linked to their respective amino acids. This activation process, called charging (Fig. 8.2), governs by the enzymes called amino acyl tRNA synthetizes. Because there are 20 different amino acids, there must be 20 different tRNA molecules and as many different enzymes.

In theory, because there are 61 triplet codes, there could be the same number of specific tRNAs and enzymes. However, because of the ability of the third member of a codon to wobble, it is now thought that there are at least 32 different tRNAs. It is also believed that there are only 20 synthetases, one for each amino acid.

During the initial step, the amino acid is converted to an activated form, reacting with ATP to create an aminoacylicadenylic acid. A covalent linkage is formed between the 5′-phosphate group of ATP and the carboxyl end of the amino acid. This enzyme-amino acid complex then reacts with a specific tRNA molecule.

In the next step, the amino acid is transferred to the appropriate tRNA and bonded covalently to the adenine residue at the 3′ end. The charged tRNA may participate directly in protein synthesis. Aminoacyl tRNA synthetases are highly specific enzymes because

they recognize only one amino acids and only a subset of corresponding tRNAs, called is accepting tRNAs. This is a crucial point in maintaining the correctness of translation.

CYTOSKELETON

the cell sap is not a liquid but has network of many interconnected fibres and filaments having similarity with the bony skeleton of the animal body, i.e., an internal scaffolding of the cell. These thread-like structures can be seen under the electron microscope or under the fluorescence microscope by tagging them with antibodies and fluorescent dyes. These network of fibres found in a cell are known as cytoskeleton.

The fibre of the cytoskeleton extends throughout the cell having interconnection with cell membrane and cell organelles. It represents some fibrous proteins of the cytoplasm which help to maintain cell shape and give contractibility to the cell.

It also helps to facilitate communication among intracellular organelles. It also helps in cell locomotion or the movement of protoplasm, i.e., cyclosis. It also helps in the movement of cellular components like chromosomes, membranes and granules, with formation of membrane protrusions (microvilli).

Components of Cytoskeleton:

On the basis of the electron microscopically observations, cytoskeleton components can be divided into three types:

i. The thickest tubular components are known as Microtubules.

ii. The thinnest fibres are called Microfilaments.

iii. The fibres of intermediate thickness are known as Intermediate filaments.

The network of microtubules becomes denser towards the nucleus, i.e., towards the nuclear envelope and then the fibres radiate towards the surface. Microfilaments, consisting of actin fibres, were found crisscrossing the cell outline.

These, microfilaments can be seen by using antibody to actin under immunofluorescence microscopy. Microfilament bundles crossover each other and also run parallel over long distances. Sometimes these filaments pass over the nucleus.

This type of microfilament organisation is sometimes known as stress fibres. The main function of these microfilaments is to help in the communication between the main cell components.

(i) Microtubules:

Microtubules were first noted in a number of eukaryotic cells by electron microscopic observations (Fig. 18.1). It is a long rod-like structure of 25 nm diameter and up to several millimeters in length.

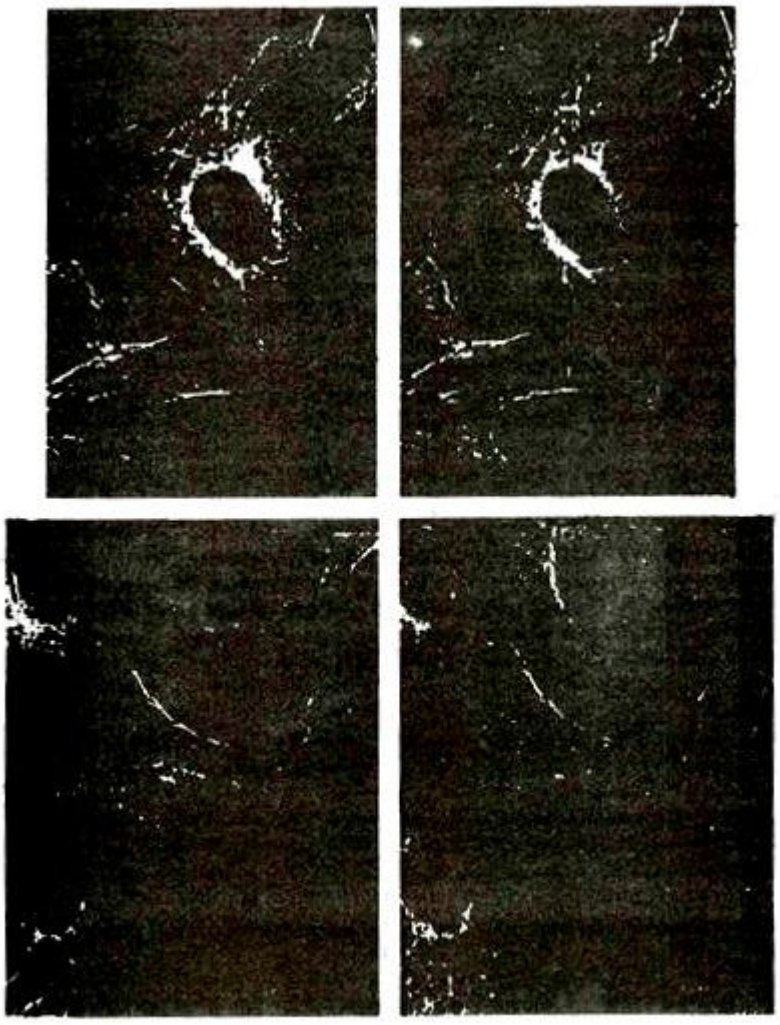

Fig. 18.1: Microtubule organisation in cultured mouse cells with the help of immunofluorescence technique.

It has two main characteristics that help to perform diverse type of functions of the cell:
i. Long rigid shape facilitates in supporting and anchoring different cellular constituents.

ii. Can generate movement in the cellular components as well as in the total cell.

Till the refinement of fixation technique in electron microscopy, microtubules were observed only in some subcellular structures like cilia, flagella, centrioles, mitotic spindle etc. using osmium tetroxide in the fixative. With the use of Glutaraldehyde in the fixative, the network of microtubules in the cytoplasm of the cell, i.e., cytoskeleton, was detected.

Electron microscopy and X-ray diffraction studies show that microtubules contain some longitudinally arranged assemblies of filaments. These filaments are known as protofilaments. About 13 protofilaments form a hollow cylinder that are recognised as microtubules (Fig. 18.2).

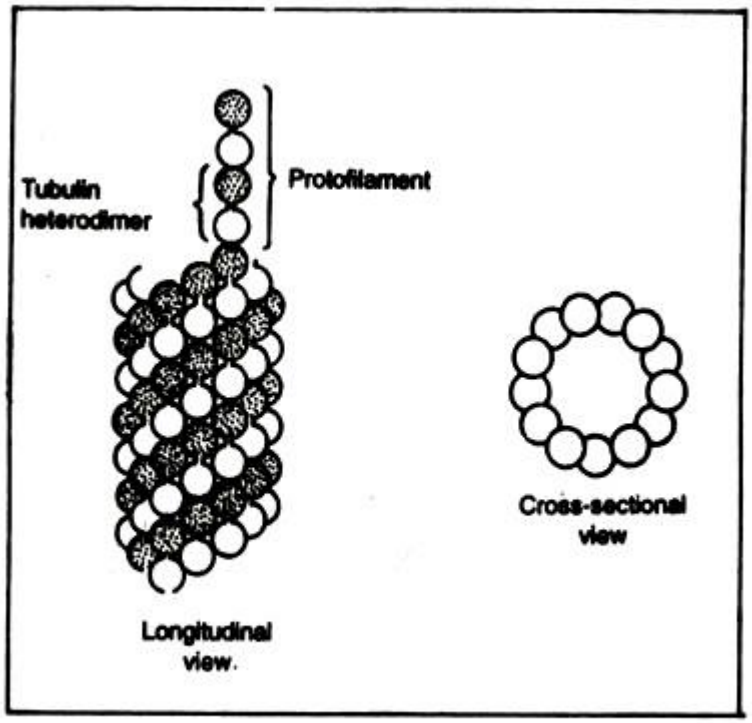

Fig. 18.2: Diagrammatic view of Microtubule substructure.

The chemical nature of these protofilaments is of tubulin molecules that are different in their amino acid sequence and are known as α and β tubulins of approximately 110,000 molecular weight. These assemblies have an outer diameter of 30 nm and an inner diameter of 14 nm with a wall thickness of 8 nm. This beaded structure of protofilaments can be observed under the electron microscope.

By treating the cells with colchicine or any inhibitors of protein synthesis or at low temperature, the organisation of the mitotic spindle was hampered (Fig. 18.3) showing the decrease in birefringence of the spindle under polarised light.

Again, with the removal of colchicine or other chemicals, the spindle is again reformed showing thereby that the microtubule organisation does not require the synthesis of new components, i.e., tubulin.

It has also been noted that microtubules remain organised when the tubulin molecules are in equilibrium with non-polymerised tubulin. So the joining and disassembly of microtubule is regulated by changes in this equilibrium.

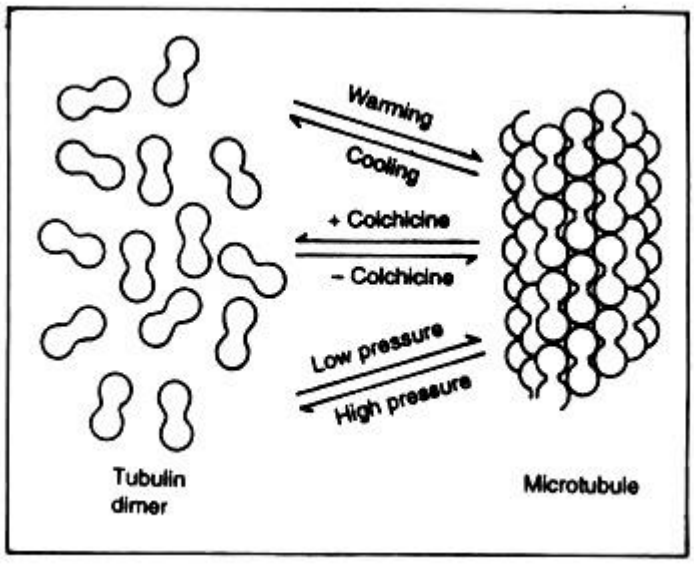

Fig. 18.3: Showing Microtubule assembly and disassembly with change of conditions.

Colchicine binds to tubulin dimers in a 1: 1 ratio in protofilaments in which the lateral tubulin-tubulin interacting sites are involved. The tubulin-colchicine complex binds only with the ends of microtubules and initiates microtubule dissociation. The tubulin-

colchicine complex activates the guanosine triphosphatase (GTPase) activity. This activation is responsible for the conformational change in the tubulin molecule.

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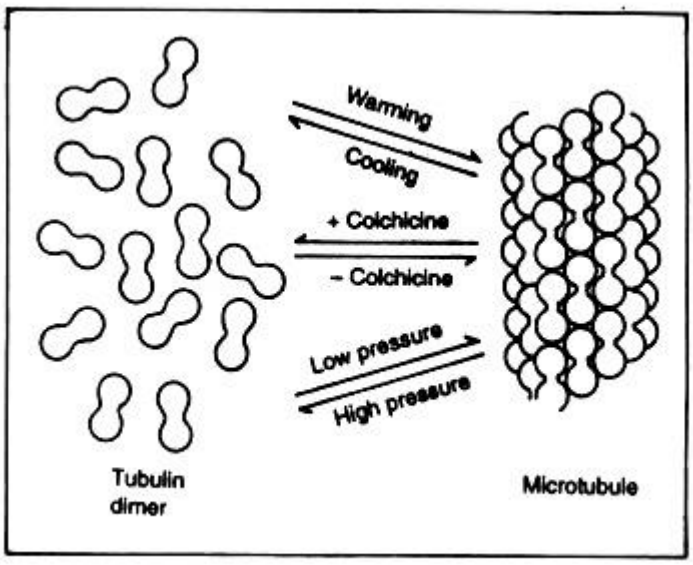

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Microtubule assembly is also inhibited by the vinca alkaloids and podophyllotoxin. In presence of these alkaloids lateral assembly of tubulin molecules GTPase of the colchicine-tubulin complex are inhibited.

But the stabilisation of micro tubular structure and the extent of microtubule assembly is increased by the interaction of taxol. Taxol is produced from the bark of Taxus baccata. Taxol promotes microtubule assembly and inhibits dissociation.

The binding of taxol to the tubulin remains on the assembled microtubule but not at the end of microtubule like that of colchicine. Thus taxol interferes with the normal course of cell division by interfering in the dynamic state of microtubules, i.e., stabilisation of microtubules.

With regard to microtubule-organisation some microtubule associated proteins and Ca^{++} ions play an important role besides tubulin and GTP. These proteins help in polymerisation of tubulin and other interactions with different cellular components.
The action of these proteins are again regulated by the activities of Protein kinases, the protein phosphorylation enzymes. Again, the activities of kinases depends on the presence of Ca^{++} and another Ca-binding protein called calmodulin.
Of the different microtubule associated proteins, two groups of high molecular weight proteins are noted. One group varies from 350KD-280KD in size. The other group includes 55 KD-80 KD. The first group of proteins has been identified as MAP-1 (350 KD) and MAP-2 (280KD).

The second group includes 'T' proteins and 'chartins'. The association of MAP proteins with microtubules has been observed by staining with fluorescent MAP antibodies. It has been remarked by Vallee, Bloom and Theurkauf (1984) that phosphorylation at MAP proteins may help in interactions with separate cytoskeletal elements in the cell.

This phosphorylation mechanism is regulated by calmodulin dependent or calmodulin independent mechanism. The presence of calmodulin is found in the site of microtubule disassembly as in the mitotic spindle.

Microtubule Organisation Centre (MTOC):

On the basis of the regular assembly and disassembly of cytoskeletal elements, it has been thought that there have some organisation centres which control the assembly process. It has been found that microtubules start assembling from a distinct structure in a highly regulated pattern.

These are clearly seen in case of flagella and cilia of lower eukaryotes and during the formation of mitotic spindles from centrioles in animal cells.

From these recognisable centres, microtubule organisation starts which is known as nucleation process. Since each centre produces many microtubules it has been assumed that each has many nucleation sites.

In this centre also, dense amorphous material is found which may have some role in the organisation of microtubules, although no direct relationship is found between the structure of the dense structure and the highly organised structure of the microtubule.

Flagella and cilia are organised from basal bodies. Each basal body contains nine sets of triplet tubules. These three tubules are known as A-tubule, B-tubule and C-tubule (Fig. 18.5). A-tubule has 13 protofilaments and B-tubule is horse shoe shaped structure having part of the wall of the A-tubule. C-tubule is also horse shoe shaped structure having part of the wall of the B-tubule.

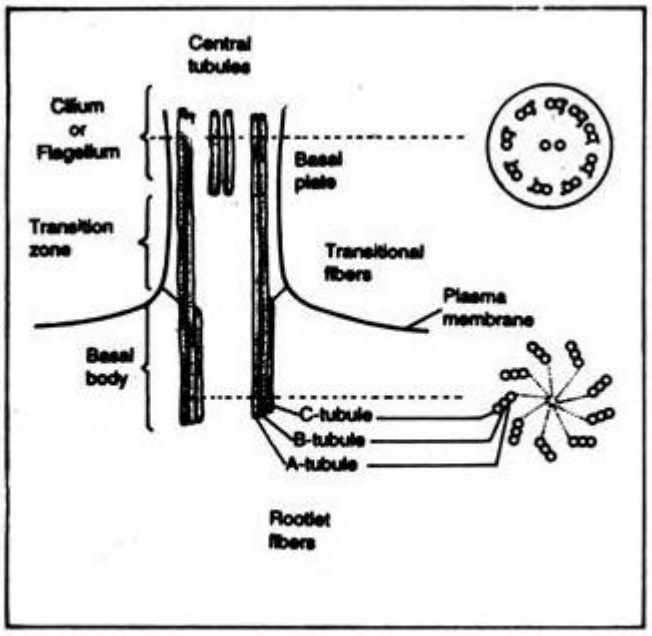

Fig. 18.5: Structure of basal body and the attachment of flagella (diagrammatic view).

The A- and B-tubules are attached with the tubules of cilia or flagella, while the C-tubules are present only in the basal body. Again, the two central tubules are present in the cilia or flagella but these are absent in the basal body.

It has been found that the organisation of cilia or flagella is controlled by centrioles present near the nucleus. Detailed mechanism for the origin of centrioles and the organisation of microtubules is not discussed here.

High voltage electron micrographic studies revealed that microtubules, microfilaments and other components form an interconnected cytoskeletal network for the cell. Besides giving architectural and mechanical support to the cell, it also helps in different metabolic processes.

One such example is in the synthesis of protein molecules that are not attached with endoplasmic reticulum. Some enzymes and substrates of the glycolysis float floated freely in the solution or loosely bound to the cytoskeletal network.

Some of the ribosomes have also been found to be attached to the cytoskeletal network in the form of multiple aggregates or polymer which are actively engaged in protein synthesis.

Any alterations in the plasma membrane can cause some response to cytoskeletal which transmits message to specific cellular target. It can also regulate the translocation of membrane components such as vesicles from the cell surface to different regions of the cell or to other organelles.

Thus the cytoskeleton has a regulatory control over the membrane process. It also gives rigidity to the cell in having connections with the plasma membrane.

(ii) Microfilaments:
The main component of these filaments is the protein actin, which is usually found in muscle. But these proteins have also been detected in many eukaryotic cells. Some actin-like proteins are also found in prokaryotic cells.

These are globular proteins of molecular weight of 42 KD. The different forms of actin (α, β and *y*) can be separated by special electrophoretic methods known as electrofocussing. The non-muscle actins differ from muscle actin by amino acid sequences.
The association of actin into cytoskeleton network has been found to be of four types:
i. Association of actin molecules into actin filaments.

ii. Association with non-actin proteins into microfilaments.

iii. Joining of microfilaments with network.

iv. Association of actin fibres with other cell components like membranes.

The role of actin as a supporting aid of various cytoplasmic structures gives an idea that the assembly of actin filaments and their associations with cell components are responsive to cellular controls. Cytochalasins metabolites from fungus Helminthosporium, have a profound effect on the actin filaments.

At low concentration of 1×10^{-9} M, it inhibits further addition of actin molecules to microfilaments. In addition, cytochalasins induce fragmentation in actin filaments.

These effects show that there may be some proteins associated with actin in the cell. Large number of such proteins have been observed that affect the state of actin. Some proteins inhibit elongation of actin filaments, others promote disassembly and nucleation.

There are some proteins which inhibit cross-linking between actin filaments or between actin and membranes. A protein which bind to the actin monomers is known as profiling of 12-15 KD in size. It inhibits ATPase activity and the polymerisation of actin.

Again, some proteins bind to the ends of the microfilaments thus inhibiting the growth of the filaments. These are known as capping proteins Fragmin, spectrin, β-actinin, Villin etc. Most of the actin-binding proteins play an important role in the binding of microfilaments to membrane.

Detailed studies have been made in the microvilli of mammalian intestine to find out the relationship between membrane and microfilaments. Longitudinal section through microvilli shows the presence of long threadlike microfilaments in the villi.

They contain actin as evidenced by the use of actin antibodies labelled with some fluorescent markers. These actin filaments are again associated with a number of proteins—Villin, Fimbrin, Calmodulin etc. These proteins help in binding of microfilaments with membrane.

(iii) Intermediate Filaments:

On the basis of size, another division of cytoskeletal components has been made which has a diameter (10 nm) smaller than that of microtubules. These filaments have a central highly conserved portion of 311-324 amino acids.

This portion has 4 segments having two a-helical structures wound around each other in each segment. This central portion is flanked by end domains—N-terminal domain and C- terminal domain (Fig. 18.4).

Four segments of different size are present in central domain of the microfilaments. These are designated as 1A, IB, 2A and 2B. 1A and 2A are short— 35 residues long. 2A and 2B are large—101 and 121 residues.

These segments are linked by some linkers, such as L_x (8-14 residues), L 12 (16 or 17 residues) and L_2 (8 residues). These central segments are then coupled with end domains which are again subdivided on the basis of charge.

Examples of Intermediate Filaments are:

Keratin fibres present in epithelial cells, Desmin filaments found in muscle cells, glial filaments and neural filaments in the cells of the nervous system, vimentin filaments present in many types of cells. Intermediate filaments help in the change of cell shape.

During culture in suspension, round-off cells cease to Synthesize intermediate filaments but when the cells firmly adhere to the substratum, synthesis of intermediate filaments go on. In case of plant cells, where cell shape is controlled by cell wall, intermediate filaments are not so common.

Ribosomes of Eukaryotic Cell:

Definition: Ribosomes are sub-microscopic, smallest, dense, membrane-less granular ribonucleoprotein organelles found in all living cells.

History:
A. Claude (1941), first observed ribosomes and called them as microsomes which were actually fragments of RER.

Robinson and Brown (1953) first discovered ribosomes, in plant cells (roots of Vicia).

Palade (1955) isolated ribosomes from animal cells and detected RNA in them.

R.B. Roberts (1958) coined the term ribosome.

Distribution and Number:
The ribosomes are present in both prokaryotic and eukaryotic cells but absent in mature RBC and sperm. In prokaryotic cells, they are found freely scattered in the cytoplasm, but in eukaryotic cells they occur free in the cytoplasmic matrix and also attached to the outer surface of the rough endoplasmic reticulum and nuclear envelope. The ribosomes are also found in the matrix of mitochondria and the stroma of plastids in the eukaryotic cells. These ribosomes are called organeller ribosomes to distinguish them from the cytoplasmic ribosomes.

Ribosomes occur singly (monosomes) or in cluster (polysomes). At the time of protein synthesis 6-8 ribosomes temporarily join with a mRNA to form a cluster called poly ribosome or polysome or ergosome.The number of ribosomes in a cell depends upon the active protein synthesis. In eukaryotic cells up to 10 million present. A plant cells may contain up to 5, 00,000 ribosomes, 10,000 – 30,000 ribosomes, forming 25% of total mass of the bacterial cell.

Types:
On the basis of sedimentation coefficient, measured in Svedberg Units or S units two types of ribosomes have been recognized – 70 S

ribosomes and 80 S ribosomes. However, ribosomes with different sedimentation value found in different phyla, e.g. 77S in fungal mitochondria and 55S in mammalian mitochondria.

1.70 S Ribosomes:
These types of ribosomes are found in prokaryotic cell such as bacteria and cyanobacteria, mitochondria and chloroplasts of eukaryotic cells. Their sedimentation coefficient is 70 S and molecular weight 2.7 x 10^6 Daltons. Each 70S ribosome is made up of two subunits the smaller 30 S subunit remains attached with larger SOS subunit like a cap.

80 S Ribosomes:
They are larger in size than 70S ribosomes. Their sedimentation coefficient is 80 S, and molecular weight 40 x 10^6 Daltons. Like 70 S ribosomes, it is also made up of two subunits – 60 S and 40 S; with 40 S placed over 60 S subunit.

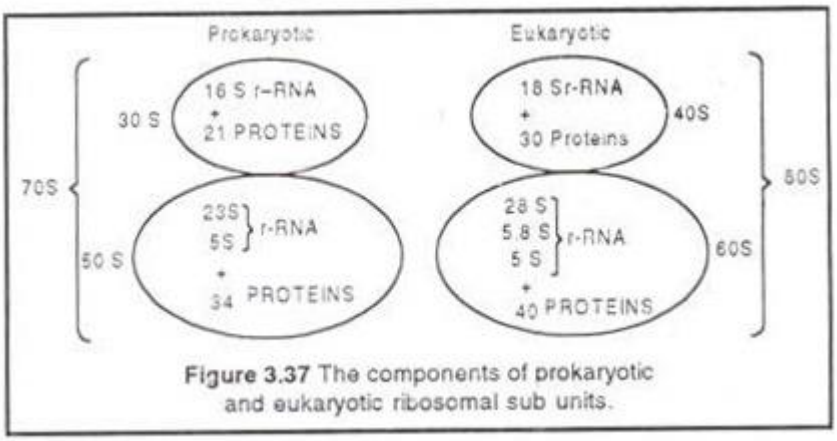

Figure 3.37 The components of prokaryotic and eukaryotic ribosomal sub units.

Ultrastructure:
Ribosomes are smallest and most abundant organelles of a cell. Each ribosome is porous, hydrated and composed of two unequal sub-units, larger one dome- shaped and the smaller one oblate – ellipsoid. The large subunit has a protuberance, a ridge and a stalk. The smaller subunit has a platform, cleft, head and base. It is about half the size of larger subunit. The smaller subunit fits over the larger one at one end like a cap (Fig. 3.38).

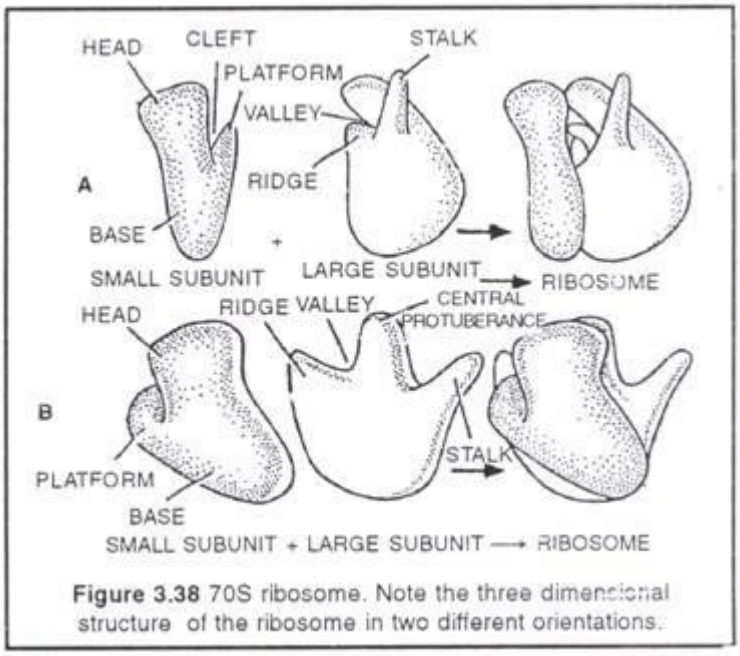

Figure 3.38 70S ribosome. Note the three dimensional structure of the ribosome in two different orientations.

The two subunits usually remain separated and come together only at the time of protein synthesis. For the union of two subunits require 0.001M of Mg^{2+} subunits dissociated below it. When Mg^{2+} concentration is above 0.0001M non-functional dimmers are formed. Each ribosome has four sites for specific functions in protein synthesis.

They are:

(i) mRNA binding site in smaller sub-unit

(ii) A-site or amino acyl-tRNA site,

(iii) P-site or peptidyl-tRNA site and

(iv) E-site or exit site to which uncharged t-RNA come before leaving the ribosome (Fig. 3.39).

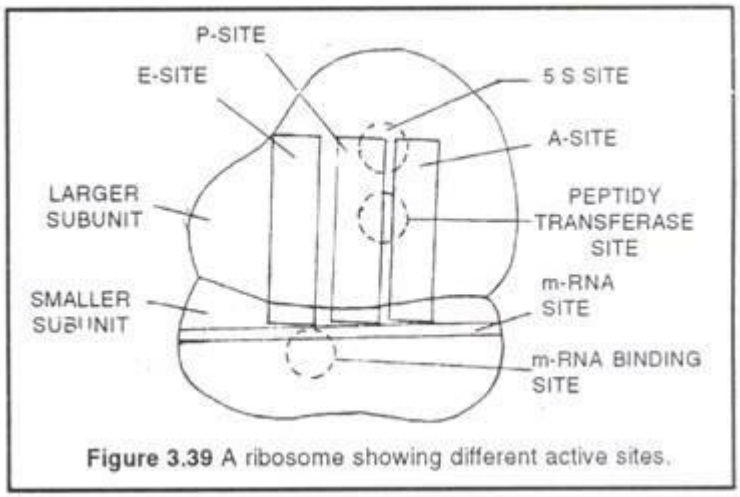

Figure 3.39 A ribosome showing different active sites.

"Chemically ribosomal," subunit consists of highly folded ribosomal RNA, (rRNA) and many attached proteins. The ratio of rRNA to protein in prokaryotic and eukaryotic ribosomes is 60:40 and 50:50 by weight respectively. The ribosomal proteins maybe basic, structural or enzymatic in function. The larger subunit of ribosome contains an important enzyme – peptidyl transferase, which brings about the formation of peptide bond. Inside the ribosome, the rRNA remains fully covered with proteins. The ribosomes are therefore, ribonucleoprotein particles (RNP).

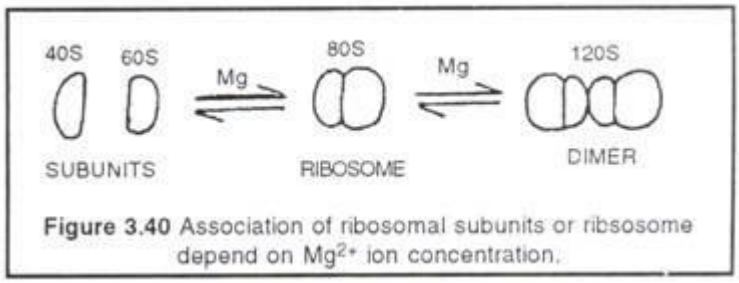

Figure 3.40 Association of ribosomal subunits or ribsosome depend on Mg^{2+} ion concentration.

Functions:

(a) As protein factories:

The ribosomes are the site of protein synthesis and also provide necessary enzymes for the same. Hence these are called "Protein Factories".

(b) Free and attached Ribosomes:

Free ribosomes synthesis structural and enzymatic proteins for use inside the cell. The attached ribosomes synthesize proteins for transport (i.e. transport proteins).

(c) Enzymes and Factors:

Ribosomes provide enzymes (e.g. peptidyl transferase) and factors for condensation of amino acids to form polypeptide.

(d) rRNA:
Ribosome contains rRNAs for providing attachment points to mRNA and tRNAs (transfer RNA).

(e) mRNA.
Ribosomes has tunnel for mRNA so that it can be translated properly.

(f) Protection.
A newly synthesized polypeptide is provided protection from cytoplasmic enzymes by enclosing it in the groove of larger subunit of ribosome till it attains secondary structure.

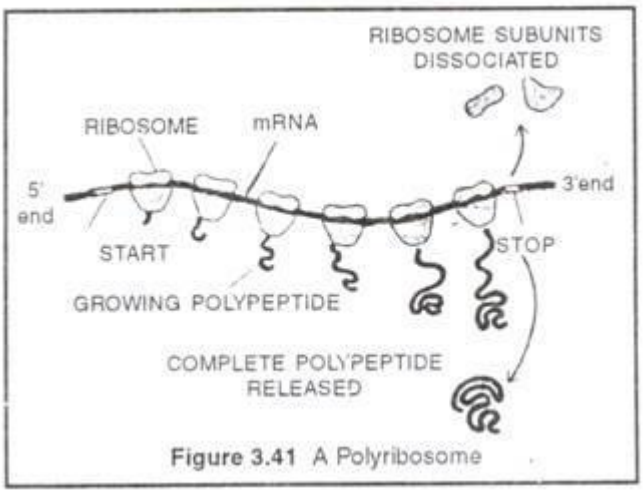

Figure 3.41 A Polyribosome